Macromolecular Research, Vol. 16, No. 5, pp 429-433 (2008)

Alginate/Carboxymethyl Scleroglucan Hydrogels for Controlled Release of Protein Drugs

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Received November 23, 2007; Revised February 16, 2008; Accepted February 19, 2008

Abstract: Alginate/carboxymethyl scleroglucan (CMSG) hydrogels were suggested as a novel carrier for the controlled release of protein drugs. The drug release characteristics of alginate hydrogels were improved by CMSG addition. Scleroglucan (Sclg) was carboxymethylated using monochloroacetic acid in aqueous alkaline medium. Alginate/CMSG hydrogels were prepared by dropping the mixture solution of alginate/CMSG into calcium chloride solution. The swelling behaviors and drug release characteristics of the hydrogels were investigated in the buffers of pH 1.2 or 7.4. As the CMSG content increased in the hydrogels, the swelling ratio of the alginate/CMSG hydrogel increased rapidly in the buffer of pH 7.4. At pH 1.2, however, the swelling ratio significantly decreased compared to that at pH 7.4. According to in vitro release tests, only 15% of ovalbumin, investigated as a model protein drug, was released from the alginate/CMSG hydrogels at pH 1.2 within 6 h. At pH 7.4, however, the drug release significantly increased due to the rapid swelling of the hydrogels. The release and swelling behaviors of the hydrogels could be controlled by changing the CMSG content in the hydrogels. These results supported the use of alginate/CMSG hydrogels as a suitable carrier for the controlled release of protein drugs in a pH responsive manner.

Keywords: alginate, beads, carboxymethylation, drug delivery systems, ovalbumin, scleroglucan.

Introduction

Administration of pharmaceutically active drugs through an oral route is the most preferred route for therapy of various diseases. However, oral drugs are readily degraded by the low pH of gastric medium in the stomach and absorbed poorly into action sites.[1,2] Therefore the drugs should be protected from harsh environment such as inactivation, degradation, and instability in the stomach.[3] The problems can be eliminated by incorporating the drugs into natural polymers.

Natural polymers, such as sodium alginate, chitosan, cellulose, gelatin, and pectin have received much attention for an oral delivery system due to their protecting effect of the drugs from harsh environment in the stomach. They have also low cytotoxicity, biocompatibility, and biodegradability. Among these polymers, sodium alginate is very promising and has been widely exploited in the design for oral delivery of protein or peptide drugs. Sodium alginate is a natural polysaccharide derived from algae and a heteropolymer made of β-$_D$-mannuronic acid (M block) and α-$_L$-guluronic acid (G block) residues in a linear form. Interestingly, it has a gel-forming property in the presence of multivalent cations such as calcium ions. The gel-formation of sodium alginate takes place mainly at junctions in the G-G sequence rich chain region known as the 'egg box junctions'.[4] The hydrogel has been used as a delivery system for live cell, protein, or peptide drugs because the gel-forming process is carried out under mild conditions as compared with the delivery system using poly(lactide-*co*-glycolide), poly(lactide-*co*-glycolide), and poly(ε-caprolactone). In particulate, the biological activity and the therapeutic effect of these drugs loaded in the hydrogel can be still retained.[5] However, the drug release from the alginate hydrogel may be limited at the absorption site due to the relatively strong ionic interaction between the carboxylic groups of alginate and calcium ions.[6] In order to eliminate the limited drug release,

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many attempts have been tried.[7-9] For example, Lin *et al.* prepared the chitosan/alginate hydrogel for oral drug delivery.[10] They reported that release characteristics of the protein drug from alginate hydrogel including chitosan could be improved with pH sensitive pattern.

In this study, a carboxymethyl scleroglucan (CMSG) was selected to improve the limited drug release from the alginate hydrogel. Scleroglucan (Sclg) produced by microorganisms consists of a main chain of (1→3)-β-D-glucopyranosyl units, bearing a branch chain of (1→6)-β-D-glucopyranosyl monomer.[11] Recently, Sclg has been proposed for the sustained-drug release and the pH-controlled drug delivery through an oral route.[12] CMSG, a derivative of Sclg having a carboxymethyl substituent, relatively forms a weaker hydrogel by ionic interaction with calcium ions than the alginate hydrogel. Therefore, the combination of alginate and CMSG will show the desirable swelling ratio and release profile. The aim of this study is to prepare the alginate/CMSG hydrogel for oral delivery of protein drugs. The influence of CMSG on drug release and swelling properties of the hydrogel was also investigated. Ovalbumin was used as the protein drug. It is used to induct the mucosal immunity in mucosal vaccine delivery. Thus, we selected ovalbumin as a model drug to evaluate the alginate/CMSG hydrogel as an oral drug delivery carrier.

Experimental

Materials. Sodium alginate of low viscosity (250 cps for a 2% (w/v) solution at 25 °C), ovalbumin (OVA), calcium chloride, and Bradford reagent were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Scleroglucan (Sclg) was presented from Deggusa Co. (Trostberg, Germany). All chemicals were used without further purification.

Synthesis of Carboxymethyl Scleroglucan (CMSG). CMSG was synthesized according to the procedure previously described by de Nooy *et al.* with some modifications.[11] In briefly, 1 g of Sclg was suspended in 40 mL of isopropyl alcohol and then the solution was stirred for 30 min at room temperature. After adding 4 mL of NaOH solution (10 M), the mixture was strongly stirred for 1 h. 1.8 g of monochloroacetic acid was added into the reaction mixture. The final solution was stirred for 3 h at 50 °C and then neutralized with acetic acid. After washing with aqueous methanol solution and dialysis against water, the solid product was freeze-dried. The synthesized CMSG was confirmed by the FT-IR.

Preparation of Alginate/CMSG Hydrogels. The alginate/CMSG hydrogels were prepared by dropping aqueous alginate-CMSG into calcium chloride solution. Alginate/CMSG solutions at distinct compositions (alginate:CMSG (weight-% ratio) = 2:0 (AS 0), 1.5:0.5 (AS 1), 1:1 (AS 2), 0.5:1.5 (AS 3), 0:2 (AS 4), 2:2 (AS 5), and 1.5:1.5 (AS 6)) were prepared. The prepared alginate-CMSG solutions were dropped into a stirred 0.1 M of calcium chloride solution through a syringe needle. The hydrogels were rinsed with distilled water to remove unreacted calcium chloride and then dried at 37 °C for 24 h. The hydrogels were examined using an optical digital camera (Optical zoom DSC-T1, Sony Co., Tokyo, Japan).

Swelling Properties of Alginate/CMSG Hydrogels. The swelling properties of the alginate-CMSG hydrogels were evaluated by immersing dried the hydrogels to swell in 1 mL of HCl buffer (0.1 M, pH 1.2) or PBS buffer (0.1 M, pH 7.4) at 37 °C. At specific time intervals, the immersed hydrogels were removed from the swelling buffer and excess water on the surface of the samples was absorbed with Whatman paper. The swelling (Q_s, %) were expressed according to the following equation:

$$Q_s = \frac{(W_s - W_d)}{W_d} \times 100$$

Where W_s is the weight of the swollen hydrogels and W_d is the weight of the dried hydrogels. The data was represented by the mean ± S.D. of three independent experiments.

Release Studies of OVA from the Alginate/CMSG Hydrogels. In order to prepare drug-loaded beads, OVA with a final concentration of 1% (w/v) was added to alginate/CMSG solution and the mixture was then dropped into a calcium chloride solution (0.1 M). The rest of the procedure was similar to those used in the preparation of the alginate/CMSG beads.

To investigate the release profiles of OVA from the hydrogels, dried samples were immersed in a buffer with pH value of 1.2 or 7.4 at 37 °C. At predetermined time point, 100 μL of the solution from release medium was taken out and the released drug was analyzed by the Bradford method at 595 nm using a UV spectrophotometer (UV-1201, Shimadzu, Kyoto, Japan). The percentage of cumulative amount of released OVA was calculated from the standard calibration curve prepared previously.

Results and Discussion

Characteristics of CMSG. Figure 1 shows the FT-IR spectra of native scleroglucan and CMSG. The intensity increase of the peak at 1067 cm^{-1} in CMSG indicates C-O stretch. The peak of 1605 cm^{-1} in CMSG is a characteristic band for the asymmetrical carboxylic acid stretching, suggesting that carboxymethyl groups exist on CMSG. The band at 1432 is assigned to the stretching vibration modes of the symmetrical carboxylic acid. This result shows that carboxymethyl groups were substituted to Sclg.

Characteristic of Alginate/CMSG Hydrogels. Alginate has been used to encapsulate cells and drugs since it forms an ionic hydrogel in the presence of calcium ions under mild conditions.[13] When CMSG solution was added into calcium chloride solution, hydrogel formation was observed. This

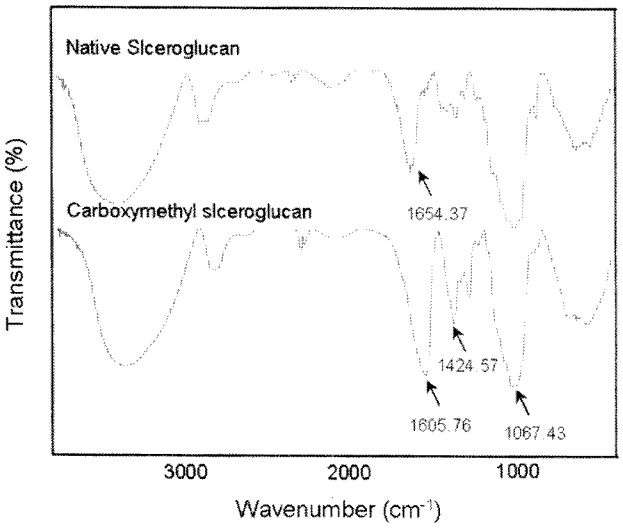

Figure 1. FT-IR spectra of slceroglucan and carboxymethylscleroglucan.

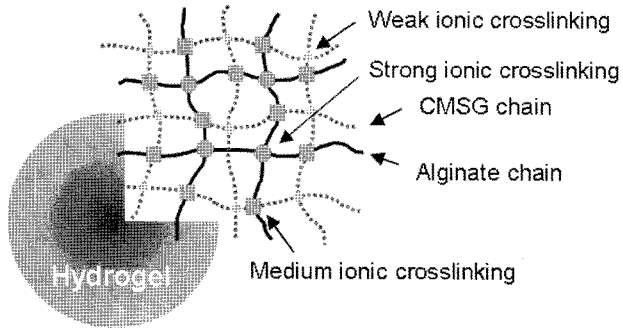

Figure 2. Schematically illustration of the physical structures of the alginate/CMSG hydrogels cross-linked with calcium ions.

phenomenon indicates that ionic interaction between the carboxylate ions of CMSG and calcium ions are presence.[14] The physical strength of the CMSG hydrogel by calcium ions was relatively weak compare with that of the alginate hydrogel (Figure 2).

Figure 3 shows the photographs of the alginate/CMSG hydrogels prepared at distinct compositions. In all the compositions, the hydrogel could be formed with a regular shape and a useful strength. The diameters of the hydrogels were approximately 0.3-0.4 mm. The photographs of the alginate/CMSG hydrogels in dried state and swelled state were shown in Figure 4. In the formation of the hydrogels using only CMSG without alginate, their shape was spherical. However, the shape with an irregular form was observed after drying. On the other hand, when alginate content in the hydrogels increased, the spontaneous formation of spherical gel with strong strength was observed. This phenomenon demonstrates that ionic interaction between CMSG and calcium ions occurs, but the cross-linking degree in the hydrogels decreases significantly as the CMSG content increased

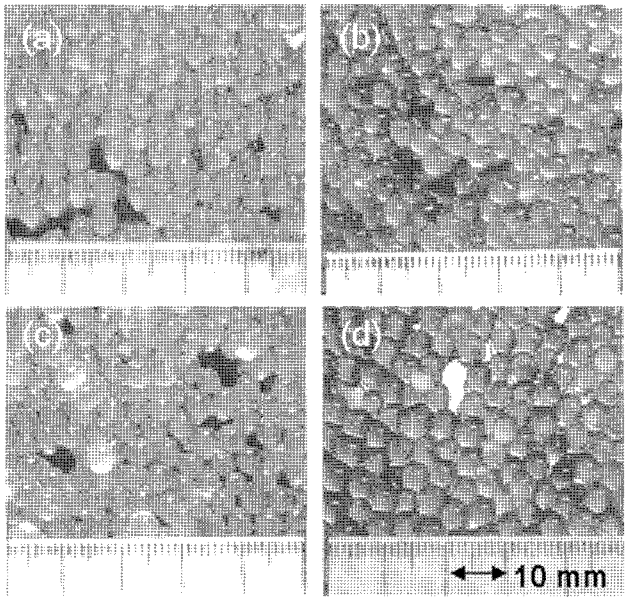

Figure 3. Photographs of the alginate/CMSG hydrogels cross-linked with calcium ions. (a) AS 0, (b) AS 1, (c) AS 2, and (d) AS 3.

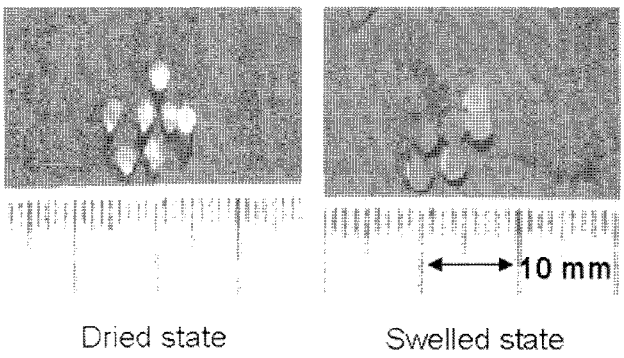

Figure 4. Photographs of the aliginate/CMSG hydrogels in dried state and swelled state.

in the alginate/CMSG hydrogel.[10] The cross-linking density was improved and controlled by addition of alginate. These results may be due to the difference of cross-linking properties by calium ions in the formation of the alginate/CMSG hydrogels. Alginate had a strong cross-linking by calium ions, while CMSG formed relatively a weakly cross-linked hydrogel.

Swelling Ability of Alginate/CMSG Hydrogels. Drug release profiles from a hydrogel depend upon their swelling behaviors. Swelling behaviors of alginate/CMSG hydrogel were investigated in the PBS buffer (pH 7.4) and HCl buffer (pH 1.2) at 37 °C. Figure 5 shows the swelling behaviors of the alginate/CMSG hydrogels. The swelling ability was dependent on the CMSG content in the hydrogels. As the CMSG content increased in the composition of the hydrogels, the rapid swelling showed in the buffer of pH 7.4. The hydrogels of AS 1 and 2 attained to maximum swelling per-

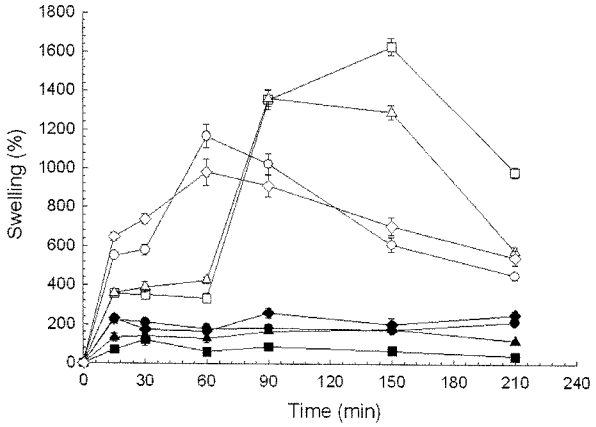

Figure 5. Swelling behavior of the alginate/CMSG hydrogels for AS 0 (square), AS 1 (triangle), AS 2 (circle), and AS 3 (diamond) at 37 °C in the buffer of pH 1.2 (closed symbol) and 7.4 (open symbol).

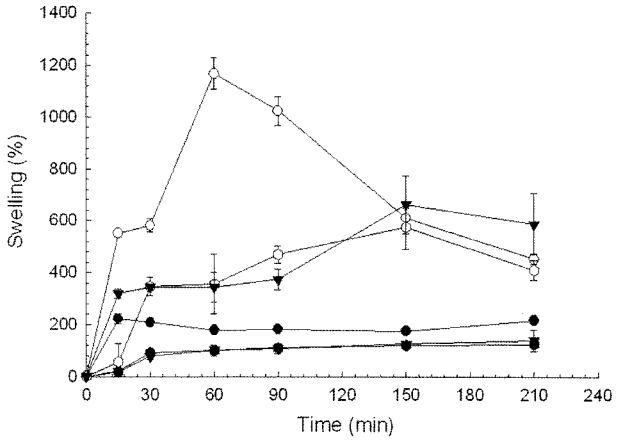

Figure 6. Swelling behavior of the alginate/CMSG hydrogels for AS 2 (triangle), AS 5 (hexagon), and AS 6 (diamond) at 37 °C in the buffer of pH 1.2 (closed symbol) and 7.4 (open symbol).

cent of 982.3 ± 58.5% and 1167.3 ± 60.8% within only 1 h. After attaining maximum swelling percent, the hydrogels began to lose weight and finally dissolve. The hydrogels of AS 4 exhibited maximum swelling percent of 1630 ± 42.0% for 150 min in the buffer of pH 7.4. These swelling behaviors may be explained as following. It is because CMSG is attributed to the poor mechanical strength of the hydrogels by their weak cross-linking network.[15] On the other hand, in the buffer of pH 1.2, the swelling percent of the hydrogels showed the significant difference as compared to those in the buffer of pH 7.4. At pH 1.2, the hydrogels swelled rapidly within first 15 min and then did not swell further. The present of CMSG in the hydrogels may be attributed to high swelling percent and it affects the mechanical strength of the hydrogel. In the design of oral delivery system for peptide or protein drugs, pH responsive hydrogels have attracted increasing attention because drugs administered orally are absorbed through the small intestine. In this study, the swelling of the alginate/CMSG hydrogel in the stomach can be minimal and thus the drug release can also be minimal. As the hydrogels passed down the intestine tract, the release ratio of the drugs from the hydrogels will increase with pH responsive pattern. Therefore, the alginate/CMSG hydrogels are a suitable carrier for the intestinal delivery of drugs through an oral route.[4] Figure 6 shows swelling behaviors of the hydrogels prepared with the alginate-to-CMSG weight ratio of 1:1. In the buffer of pH 7.4, as the total concentration of alginate-CMSG increased, the swelling ratio of the hydrogels decreased. The hydrogels of AS 2 with the alginate-CMSG composition of 1%:1% (w/v) showed rapid swelling ratio and stared to disintegrate within 1 h in the buffer of pH 7.4. In contrast, the hydrogels of AS 5 and 6 with the alginate-CMSG composition of 1.5%:1.5% (w/v) and 2%:2% (w/v) swelled slowly and attained to the maximum swelling percent at 150 min. These results may be due to the tight ionic cross-link between alginate and calcium ions.[10] Swelling behaviors of the alginate/CMSG hydrogels could be controlled by changing the content of alginate and CMSG, and it may be influenced on drug release from the hydrogels.

Release Profiles of OVA from Alginate/CMSG Hydrogels. Figure 7 shows release profiles of OVA from the alginate/CMSG hydrogels. As shown in the figure, in the buffer of pH 1.2, the cumulative amount of OVA released from the hydrogels was less than 15% within 6 h. In contrast, as the CMSG content in the hydrogels increased, the OVA amount released from the hydrogels significantly increased in the buffer of pH 7.4. After 1 h, the hydrogels of AS 4 released 47.3 ± 7.5% of total OVA loaded in the hydrogels, but the amount of OVA released from the hydrogels of AS 1 was approximately 100% for the same time. This result shows that the drug release behaviors from the hydrogels are

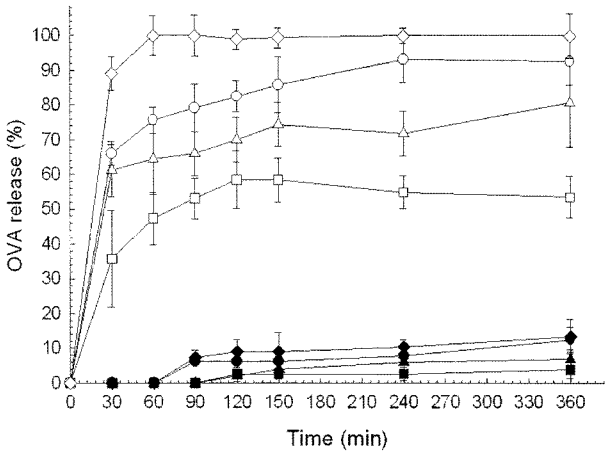

Figure 7. Release properties of OVA from the alginate/CMSG hydrogels for AS 0 (square), AS 1 (triangle), AS 2 (circle), and AS 3 (diamond) at 37 °C in the buffer of pH 1.2 (closed symbol) and 7.4 (open symbol).

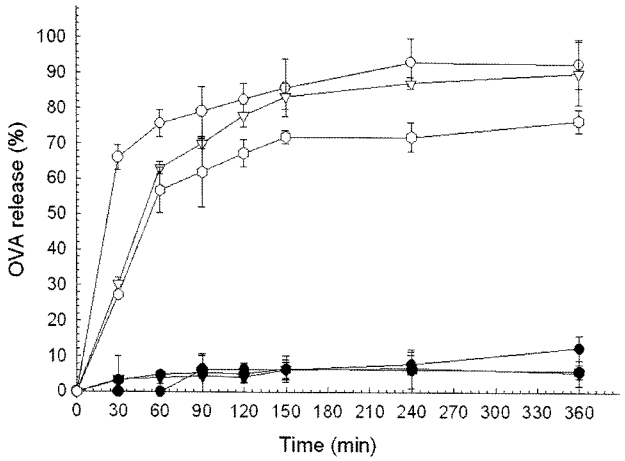

Figure 8. Release properties of OVA from the alginate/CMSG hydrogels for AS 2 (circle), AS 5 (triangle), and AS 6 (hexagon) at 37 °C in the buffer of pH 1.2 (closed symbol) and 7.4 (open symbol).

matched to the swelling behaviors of the hydrogels.

OVA release profiles from the hydrogels prepared with the alginate-to-CMSG weight ratio of 1:1 were shown in Figure 8. In the buffer of pH 7.4, as the total concentration of alginate-CMSG increased, the release of OVA comparatively decreased. The hydrogels of AS 5 and 6 with the alginate-CMSG composition of 1.5%:1.5% and 2%:2% released 83.4 ± 3.7% and 71.9 ± 1.8% of the total of OVA loaded in the hydrogels within 150 min. The hydrogel with the alginate-CMSG composition of 1%:1% exhibited more the rapid release characteristics within the same time. OVA release from the hydrogels could be controlled by changing the content of alginate and CMSG in the hydrogels. As our expectation, the limited drug release properties from the alginate hydrogels could be improved by addition of CMSG. Recently, George and Abraham reported the pH sensitive alginate-guar gum hydrogel for the controlled delivery of protein drugs.[16] The alginate-guar gum hydrogel was required the use of cross-linking procedures by glutaraldehyde treatment. However, the use of cross-linking agents may lead to toxic side effects. In contrast, the hydrogel presented in this study was prepared under mild condition and it is biocompatible without toxic side effects. In the design of oral delivery system for peptide or protein drugs, pH responsive hydrogels have attracted increasing attention because drugs administered orally are absorbed through the small intestine. In this study, the swelling of the alginate/CMSG hydrogel in the stomach can be minimal and thus the drug release can also be minimal. As the hydrogels passed down the intestine tract, the release ratio of the drugs from the hydrogels will increase with pH responsive pattern. Therefore, the alginate/CMSG hydrogels are a suitable carrier for the intestinal delivery of drugs through an oral route.

Conclusions

It is concluded that the alginate/CMSG hydrogels cross-linked calcium can be a suitable delivery carrier for a protein drug. The main advantage of this system is to improve the limited swelling and release properties of the alginate hydrogels. In this study, the swelling behaviors were dependent on the content of CMSG in the alginate/CMSG hydrogels and the release of OVA from the alginate/CMSG hydrogels was controlled by the changing the content of CMSG. Finally, these results clearly suggest that the hydrogel prepared with alginate and CMSG is a good candidate as a protein delivery system.

Acknowledgements. This research was supported by the Program for the Training of Graduate Students in Regional Innovation which was conducted by the Ministry of Commerce, Industry and Energy of the Korea Government.

References

(1) R. Pandey, Z. Ahmad, and S. Sharma, *Int. J. Pharm.*, **301**, 268 (2005).
(2) C. M. Lee, H. C. Lee, and K. Y. Lee, *J. Bionsci. Bioeng.*, **100**, 255 (2005).
(3) L. T. Sarah and A. D. Tejal, *Drug Discovery Today*, **10**, 909 (2005).
(4) P. Matricardi, I. Onorati, T. Coviello, and F. Alhaique, *Int. J. Pharm.*, **316**, 21 (2006).
(5) M. A. Wheatley, M. Chang, E. Park, and R. J. Langer, *Appl. Polym. Sci.*, **43**, 2123 (1991).
(6) S. K. Bajpai, S. K. Sazxena, and S. Sharma, *React. Funct. Polym.*, **66**, 431 (2005).
(7) C. J. Knill, J. F. Kennedy, J. Mistry, M. Miraftab, G. Smart, M. R. Groocock, and H. J. Williams, *Carbohyd. Polym.*, **55**, 65 (2004).
(8) M. Livnat, R. Beyar, and D. J. Seliktar, *J. Biomed. Mater. Res. A*, **75**, 710 (2005).
(9) F. L. Mi, H. F. Liang, Y. C. Wu, Y. S. Lin, T. F. Yang, and H. W. Sung, *J. Biomater. Sci. Polym. Ed.*, **16**, 1333 (2005).
(10) Y. H. Lin, H. F. Liang, C. K. Chung, M. C. Chen, and H. W. Sung, *Biomaterials*, **26**, 2105 (2005).
(11) A. E. J. de Nooy, V. Rori, G. Masci, M. Dentini, and V. Crescenzi, *Carbohyd. Res.*, **324**, 116 (2000).
(12) E. Aasprong, O. Smidsrod, and B. T. Stokke, *Biomacromolecules*, **4**, 914 (2003).
(13) G. Orive, S. K. Tam, J. L. Pedraz, and J. P. Halle, *Biomaterials*, **27**, 3691 (2006).
(14) E. Racope, J. F. Thibault, J. C. E. Rietsma, and W. Pilnik, *Biopolymer*, **28**, 1435 (1989).
(15) T. Coviello, M. Grassi, G. Rambone, and F. B. Alhaique, *Biomaterials*, **22**, 1899 (2001).
(16) M. George and T. E. Abraham, *Int. J. Pharm.*, **335**, 123 (2007).